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L2 ANSWER 1 OF 14 MEDLINE on STN DUPLICATE 1
AN 2003008806 MEDLINE
DN 22403029 PubMed ID: 12515289
TI Isolation and characterization of human monoclonal autoantibodies to glutamic acid decarboxylase.
AU Hayakawa N; Premawardhana L D K E; Powell M; Masuda M; Arnold C; Sanders J; Evans M; Chen S; Jaume J C; Baekkeskov S; Smith B Rees; Furmaniak J
CS FIRS Laboratories, RSR Ltd, Parc Ty Glas, Llanishen, Cardiff CF14 5DU, UK.
NC DK47043 (NIDDK)
EY00364 (NEI)
SO AUTOIMMUNITY, (2002 Aug) 35 (5) 343-55.
Journal code: 8900070. ISSN: 0891-6934.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030108
Last Updated on STN: 20030503
Entered Medline: 20030502

L2 ANSWER 2 OF 14 MEDLINE on STN DUPLICATE 2
AN 2003008805 MEDLINE
DN 22403028 PubMed ID: 12515288
TI Immune reactivity to GAD25 in type 1 diabetes mellitus.
AU Chessler Steven D; Hampe Christiane S; Ortqvist Eva; Simonson William T; Bekris Lynn
CS Robert H. Williams Laboratory, Department of Medicine, HSB K-161, Box 357710, University of Washington, Seattle, WA 98195-7710, USA.. chessler@u.washington.edu
NC DK02944 (NIDDK)
DK17047 (NIDDK)
DK26190 (NIDDK)
DK42654 (NIDDK)
DK53004 (NIDDK)
SO AUTOIMMUNITY, (2002 Aug) 35 (5) 335-41.
Journal code: 8900070. ISSN: 0891-6934.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030108
Last Updated on STN: 20030503
Entered Medline: 20030502

L2 ANSWER 3 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:520791 BIOSIS
DN PREV200000520791
TI ***Insulin*** -dependent diabetes mellitus-specific ***chimeric*** polypeptides.
AU Powers, Alvin C. (1)
CS (1) Brentwood, TN USA
ASSIGNEE: Vanderbilt University
PI US 6060593 May 09, 2000
SO Official Gazette of the United States Patent and Trademark Office Patents, (May 9, 2000) Vol. 1234, No. 2, pp. No pagination. e-file.
ISSN: 0098-1133.
DT Patent
LA English

L2 ANSWER 4 OF 14 MEDLINE on STN DUPLICATE 3

AN 2000324811 MEDLINE
DN 20324811 PubMed ID: 10868936
TI Maturation of the humoral autoimmune response to epitopes of ***GAD*** in preclinical childhood type 1 diabetes.
AU Bonifacio E; Lampasona V; Bernasconi L; Ziegler A G
CS Istituto Scientifico San Raffaele, Milan, Italy.. bonifacio.ezio@hsr.it
SO DIABETES, (2000 Feb) 49 (2) 202-8.
Journal code: 0372763. ISSN: 0012-1797.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200007
ED Entered STN: 20000714
Last Updated on STN: 20000714
Entered Medline: 20000706

L2 ANSWER 5 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:283466 BIOSIS
DN PREV200000283466
TI ***Insulin*** -dependent diabetes mellitus-specific ***chimeric*** polypeptides.
AU Powers, Alvin C. (1)
CS (1) Brentwood, TN USA
ASSIGNEE: Vanderbilt University, Nashville, TN, USA
PI US 5968757 October 19, 1999
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 19, 1999) Vol. 1227, No. 3, pp. No pagination. e-file.
ISSN: 0098-1133.
DT Patent
LA English

L2 ANSWER 6 OF 14 MEDLINE on STN DUPLICATE 4
AN 2000063708 MEDLINE
DN 20063708 PubMed ID: 10594551
TI Comparative analysis of epitope recognition of glutamic acid decarboxylase (***GAD***) by autoantibodies from different autoimmune disorders.
AU Powers A C; Bavik K; Tremble J; Daw K; Scherbaum W A; Banga J P
CS Division of Endocrinology, Department of Medicine, Vanderbilt University, Department of Veterans Affairs Medical Center, Nashville, TN 37232, USA.. Al.Powers@mcmail.vanderbilt.edu
NC DK20593 (NIDDK)
SO CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1999 Dec) 118 (3) 349-56.
Journal code: 0057202. ISSN: 0009-9104.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200001
ED Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000113

L2 ANSWER 7 OF 14 MEDLINE on STN
AN 1999107526 MEDLINE
DN 99107526 PubMed ID: 9892508
TI Autoantigenic reactivity of diabetes sera with a hybrid glutamic acid decarboxylase GAD67-65 molecule GAD67(1-101)/GAD65(96-585).
AU Teoh K L; Fida S; Rowley M J; Mackay I R
CS Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia.
SO AUTOIMMUNITY, (1998) 28 (4) 259-66.
Journal code: 8900070. ISSN: 0891-6934.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990426
Last Updated on STN: 19990426
Entered Medline: 19990413

L2 ANSWER 8 OF 14 MEDLINE on STN DUPLICATE 5
AN 1998366731 MEDLINE
DN 98366731 PubMed ID: 9703171
TI Humoral and cellular immune parameters before and during immunosuppressive therapy of a patient with stiff-man syndrome and ***insulin*** dependent diabetes mellitus.
AU Hummel M; Durinovic-Bello I; Bonifacio E; Lampasona V; Endl J; Fessele S; Then Bergh F; Trenkwalder C; Standl E; Ziegler A G
CS Diabetes Research Institute and 3rd Medical Department, Academic City Hospital, Munchen-Schwabing, Munich, Germany.
SO JOURNAL OF NEUROLOGY, NEUROSURGERY AND PSYCHIATRY, (1998 Aug) 65 (2)
204-8.
Journal code: 2985191R. ISSN: 0022-3050.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)

LA English
FS Priority Journals
EM 199808
ED Entered STN: 19980828
Last Updated on STN: 20000303
Entered Medline: 19980820

L2 ANSWER 9 OF 14 MEDLINE on STN DUPLICATE 6
AN 97397290 MEDLINE
DN 97397290 PubMed ID: 9253351
TI Human B cells secreting immunoglobulin G to glutamic acid decarboxylase-65 from a nondiabetic patient with multiple autoantibodies and Graves' disease: a comparison with those present in type 1 diabetes.
AU Tremble J; Morgenthaler N G; Vlug A; Powers A C; Christie M R; Scherbaum W A; Banga J P
CS Department of Medicine, King's College School of Medicine, London, United Kingdom.
NC DK-20593 (NIDDK)
SO JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, (1997 Aug) 82 (8)
2664-70.
Journal code: 0375362. ISSN: 0021-972X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199708
ED Entered STN: 19970908
Last Updated on STN: 19970908
Entered Medline: 19970828

L2 ANSWER 10 OF 14 MEDLINE on STN DUPLICATE 7
AN 97031369 MEDLINE
DN 97031369 PubMed ID: 8877294
TI Diagnostic sensitivity of immunodominant epitopes of glutamic acid decarboxylase (GAD65) autoantibodies in childhood IDDM.
AU Falorni A; Ackefors M; Carlberg C; Daniels T; Persson B; Robertson J; Lernmark A
CS Department of Molecular Medicine, Karolinska Institute, Stockholm, Sweden.
NC DK 42654 (NIDDK)
SO DIABETOLOGIA, (1996 Sep) 39 (9) 1091-8.
Journal code: 0006777. ISSN: 0012-186X.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199702
ED Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970206

L2 ANSWER 11 OF 14 MEDLINE on STN DUPLICATE 8
AN 96133013 MEDLINE
DN 96133013 PubMed ID: 8543838
TI Glutamic acid decarboxylase autoantibodies in stiff-man syndrome and ***insulin*** -dependent diabetes mellitus exhibit similarities and differences in epitope recognition.
AU Daw K; Ujihara N; Atkinson M; Powers A C
CS Department of Medicine, Vanderbilt University, Nashville, TN 37232, USA.
NC BRSG RR05424 (NCRR)
DK20593 (NIDDK)
R01DK43736 (NIDDK)
SO JOURNAL OF IMMUNOLOGY, (1996 Jan 15) 156 (2) 818-25.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199602
ED Entered STN: 19960227
Last Updated on STN: 20000303
Entered Medline: 19960214

L2 ANSWER 12 OF 14 MEDLINE on STN DUPLICATE 9
AN 97060884 MEDLINE
DN 97060884 PubMed ID: 8904930
TI Murine monoclonal glutamic acid decarboxylase (***GAD***)65 antibodies recognize autoimmune-associated ***GAD*** epitope regions targeted in patients with type 1 diabetes mellitus and stiff-man syndrome.
AU Ziegler B; Schlosser M; Luhder F; Strebelow M; Augstein P; Northemann W; Powers A C; Ziegler M
CS Institute of Diabetes Gerhardt Katsch Karlsburg, Germany.
NC DK20593 (NIDDK)
R01DK43736 (NIDDK)
SO ACTA DIABETOLOGICA, (1996 Sep) 33 (3) 225-31.
Journal code: 9200299. ISSN: 0940-5429.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 199702
ED Entered STN: 19970305
Last Updated on STN: 20000303
Entered Medline: 19970218

L2 ANSWER 13 OF 14 MEDLINE on STN DUPLICATE 10
AN 95163801 MEDLINE
DN 95163801 PubMed ID: 7532143
TI Two distinct glutamic acid decarboxylase auto-antibody specificities in IDDM target different epitopes.
AU Daw K; Powers A C
CS Department of Medicine, Vanderbilt University, Nashville, Tennessee 37232.
NC DK-20593 (NIDDK)
R01-DK-43736 (NIDDK)
RR-05424 (NCRR)
SO DIABETES, (1995 Feb) 44 (2) 216-20.
Journal code: 0372763. ISSN: 0012-1797.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199503
ED Entered STN: 19950404
Last Updated on STN: 19960129
Entered Medline: 19950321

L2 ANSWER 14 OF 14 MEDLINE on STN DUPLICATE 11
AN 93219427 MEDLINE
DN 93219427 PubMed ID: 8464926
TI Association of ***GAD*** -65, but not of ***GAD*** -67, with the Golgi complex of transfected Chinese hamster ovary cells mediated by the N-terminal region.
AU Solimena M; Aggujaro D; Muntzel C; Dirkx R; Butler M; De Camilli P; Hayday A
CS Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT 06510.
NC 43708 (NIAID)
AI 30248-01 (NIDDK)
DK 43078-01
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
AMERICA, (1993 Apr 1) 90 (7) 3073-7.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199305
ED Entered STN: 19930521
Last Updated on STN: 19930521
Entered Medline: 19930504

=> d 14 abs

L2 ANSWER 14 OF 14 MEDLINE on STN DUPLICATE 11
AB Glutamic acid decarboxylase (***GAD***) is the enzyme responsible for synthesis of the neurotransmitter gamma-aminobutyric acid in neurons and pancreatic beta cells. It is represented by two isoforms, ***GAD*** -65 and ***GAD*** -67, which are the products of two different genes and differ substantially only at their N-terminal regions. ***GAD*** -65 is a dominant autoantigen in stiff-man syndrome and ***insulin*** -dependent diabetes mellitus. In neurons and beta cells, ***GAD*** is concentrated around synaptic vesicles and synaptic-like microvesicles, respectively, as well as in the area of the Golgi complex. The mechanisms responsible for specific targeting of ***GAD*** to these organelles are not yet understood. The elucidation of the mechanism of subcellular targeting of ***GAD*** may be relevant to understanding its role as an autoantigen. In this study, the cloned genes for ***GAD*** -65 and ***GAD*** -67 were expressed separately in Chinese hamster ovary (CHO) cells and COS cells. While ***GAD*** -67 had a diffuse cytoplasmic localization, ***GAD*** -65 had a punctate distribution, with most of the immunoreactivity being concentrated in the area of the Golgi complex. A ***chimeric*** protein in which the 88 N-terminal amino acids of ***GAD*** -67 were replaced by the 83 N-terminal amino acids of ***GAD*** -65 was targeted to the Golgi complex, indicating that the N-terminal region of ***GAD*** -65 contains a targeting signal sufficient for directing the remaining portion of the molecule, highly similar in ***GAD*** -65 and ***GAD*** -67, to the Golgi complex-associated structures.

=> d 1-13 abs

L2 ANSWER 1 OF 14 MEDLINE on STN DUPLICATE 1
AB Production of human monoclonal autoantibodies to glutamic acid decarboxylase M(r) 65,000 (GAD65), characterization of their isotype, binding affinity, V region sequences and competition with autoantibodies in patients' sera is described. Lymphocytes from a patient with Addison's disease who had GAD65 autoantibodies without diabetes were immortalised

and fused to a mouse/human hybridoma. In addition, mouse monoclonal antibodies to GAD65 were produced using standard techniques. F(ab')2S from our monoclonals and the GAD6 mouse monoclonal were used in competition with intact monoclonals and sera from diabetic patients for binding to 125I-labelled GAD65 (amino acids 46-586). Reactivities of the human monoclonals with ***GAD*** 65,000/67,000 M(r) ***chimeras*** were also studied. Variable region genes of human monoclonals were sequenced and analysed. The human monoclonals (n = 3) had affinity constants for GAD65 of 2.2 x 10(9), 5.8 x 10(9), 1.3 x 10(10) mol/l(-1); affinities of the mouse monoclonals (n = 5) ranged from 1.1 x 10(8) to 5.4 x 10(10) mol/l(-1). The binding of each of the human monoclonals was inhibited by GAD6 F(ab')2 and the binding of GAD6 antibody was inhibited by the human monoclonal F(ab')2S suggesting that the epitopes for these antibodies were overlapping. Studies with GAD65/GAD67 ***chimeras*** indicated that the human monoclonals reacted with C-terminal epitopes. The human monoclonals, GAD6 and 3/5 mouse monoclonals inhibited serum autoantibody binding to 125I-labelled GAD65. Overall, the human monoclonals were of high affinity, reacted with C-terminal epitopes and showed evidence of antigen driven maturation; they represented only a proportion of the repertoire of autoantibodies to GAD65 in the donor's serum and in the sera of patients with type-1 diabetes.

L2 ANSWER 2 OF 14 MEDLINE on STN DUPLICATE 2

AB While both isoforms of glutamic acid decarboxylase (***GAD***) function as important autoantigens in autoimmune diabetes mellitus-GAD65 in humans and GAD67 in the NOD mouse-GAD67 is not synthesized in human pancreatic islets and is thought not to be an autoantigen in human diabetes. We have recently shown, however, that human islets contain a GAD67 splice variant: GAD25. Given the evidence that GAD67 could be a key diabetogenic autoantigen in the NOD mouse and the high prevalence of GAD65 autoantibodies in human type 1 diabetes, it became important to ask whether there is also immune reactivity to GAD25 in type 1 diabetes-possibly implicating it in the pathogenesis of the disease-and whether GAD25 reactivity could, like GAD65 reactivity, function as a clinically useful marker for the disease. We also hypothesized that the presence of autoantibodies to the smaller splice variant could be a cause of the up to 30% prevalence of GAD67 autoreactivity associated with type 1 diabetes. We therefore analyzed GAD25 reactivity in 105 newly-diagnosed children with type 1 diabetes and 74 control subjects. While 14 (13%) of the diabetic subjects were positive for GAD67 autoantibodies, only 3 (3%) were positive for GAD25 reactivity, none of which were GAD67 antibody-positive. Analysis of reactivity to a GAD67 ***chimera*** was consistent with GAD67 binding activity being due to cross-reactive GAD65 antibodies. Immunostaining confirmed the presence of GAD25 in human islets, revealing GAD25-positive cells to be sparse. Our results indicate that autoreactivity to GAD25 is rare in newly diagnosed type 1 diabetes and does not underlie GAD67 reactivity.

L2 ANSWER 3 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AB The present invention provides a ***chimeric*** polypeptide comprising an epitope of GAD65 protein and a structural region comprising a polypeptide of the ***GAD*** family, wherein the ***chimeric*** polypeptide is a more specific diagnostic for ***insulin*** dependent diabetes mellitus than intact GAD65 and produces fewer false positives than intact GAD65. The invention further provides a method of screening a subject for risk of developing IDDM, comprising contacting the ***chimeric*** polypeptide of claim 1 with a biological sample containing antibodies from the subject and detecting binding between an antibody in the biological sample and the ***chimeric*** polypeptide, the detection of binding indicating the subject is at risk of developing IDDM.

L2 ANSWER 4 OF 14 MEDLINE on STN DUPLICATE 3

AB ***GAD*** is a major target of autoimmunity in preclinical type 1 diabetes. Here we examine the maturation of the humoral response to ***GAD*** epitopes sequentially from birth to diabetes onset or current follow-up in 29 ***GAD*** antibody (GADA)+ offspring of parents with diabetes from the BABYDIAB Study. Antibodies were measured against GAD65, GAD67, and GAD65/67 ***chimeras*** by radiobinding assay. In 28 of 29 offspring, the first GADAs contained reactivity against epitopes within GAD65 residues 96-444, suggesting that the middle GAD65 region is a primary target of ***GAD*** humoral autoimmunity. In 7 of these 28 offspring, initial antibody reactivity was against all epitope regions tested (middle GAD65, COOH-terminal GAD65 residues 445-585, NH2-terminal GAD65 residues 1-95, and GAD67); in 16 offspring, reactivity was to middle and COOH-terminal GAD65 epitopes, and in 5 offspring, reactivity was only to the middle GAD65 epitopes. The single offspring without middle GAD65 reactivity had antibodies to the NH2-terminal epitopes in the absence of all other islet autoimmunity. Subsequent GADA epitope spreading was frequent and seen in 10 of 15 offspring with informative follow-up samples. Spreading was mostly (eight cases) to NH2-terminal GAD65 epitopes. In two offspring, spreading to new epitopes was found when antibody titers to GAD65 and early epitopes were declining, suggesting determinant-specific regulation of the humoral response. None of the GADA reactivities nor any changes in reactivity over time were specifically associated with diabetes onset. The findings suggest that the humoral autoimmune response to ***GAD*** found in childhood is dynamic, is initially against epitopes within the middle portion of GAD65, and spreads to epitopes in other regions of GAD65 and GAD67.

L2 ANSWER 5 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AB The present invention provides a ***chimeric*** polypeptide comprising an epitope of GAD65 protein and a structural region comprising a polypeptide of the ***GAD*** family, wherein the ***chimeric*** polypeptide is a more specific diagnostic for ***insulin*** dependent diabetes mellitus than intact GAD65 and produces fewer false positives than intact GAD65. The invention further provides a method of screening a subject for risk of developing IDDM, comprising contacting the ***chimeric*** polypeptide of claim 1 with a biological sample containing antibodies from the subject and detecting binding between an antibody in the biological sample and the ***chimeric*** polypeptide, the detection of binding indicating the subject is at risk of developing IDDM.

L2 ANSWER 6 OF 14 MEDLINE on STN DUPLICATE 4

AB Autoantibodies to ***GAD*** , an important marker of the autoimmune process in type I or ***insulin*** -dependent diabetes mellitus (IDDM), are also found in non-diabetic individuals with autoimmune polyendocrine syndrome type 1 (APS1), APS2, and stiff man syndrome (SMS). Most IDDM sera contain two distinct ***GAD*** antibody specificities, one of which targets an epitope region in the middle-third of GAD65 (IDDM-E1; amino acids 221-359) and one of which targets the carboxy-third of GAD65 (IDDM-E2; amino acids 453-569). Using 11 ***chimeric*** GAD65/GAD67 proteins to maintain conformation-dependent epitopes of GAD65, we compared the humoral repertoire of IgG antibodies from an individual with APS2-like disease (b35, b78, and b96) and MoAbs from an IDDM patient (MICA-2, MICA-3, and MICA-4). Neither the APS2 IgG antibodies nor the IDDM MoAbs bind the amino-terminal third of GAD65, but instead target the carboxy-terminal two-thirds of GAD65. Amino acids 270-359 (IDDM-E1) are targeted by one APS2 IgG antibody and MICA-4, while two other APS2 IgG antibodies, MICA-2 and MICA-3, target amino acids 443-585 (IDDM-E2). Using GAD65/67 ***chimera*** that span the IDDM-E2 region, we found that MICA-2 binds amino acids 514-528 of GAD65, but two APS2 IgG antibodies require this region and amino acids 529-570. In contrast, the binding of MICA-3 requires two discontinuous amino acid segments of GAD65 (452-513 and 528-569), but not amino acids 514-528. These results indicate that there are both similarities and differences in the humoral response to GAD65 in APS2 and IDDM.

L2 ANSWER 7 OF 14 MEDLINE on STN

AB Glutamic acid decarboxylase (***GAD***) is a major autoantigen in ***insulin*** -dependent diabetes mellitus (IDDM). Two ***GAD*** isoforms exist, GAD65 and GAD67, which differ mostly in the first 100 amino acids of the amino terminus. IDDM sera are predominantly reactive with GAD65 but autoepitopes have been localised only to regions of GAD65 highly homologous with GAD67. In this study we investigated the contribution of the amino terminus to the IDDM epitope on GAD65, in order to test whether this region of ***GAD*** could explain the difference in reactivity between GAD65 and GAD67. A recombinant hybrid ***GAD*** molecule consisting of amino acids 1-101 of GAD67 and 96-585 of GAD65 was constructed and a truncated GAD65 was also constructed consisting of amino acids 98-585 of GAD65. The reactivity with the hybrid ***GAD*** molecule, GAD65 and GAD67, and truncated GAD65 was examined by radioimmunoprecipitation using 50 IDDM sera with known reactivity to purified porcine brain ***GAD*** . Over 90% of the IDDM sera were reactive with the hybrid ***GAD*** molecule confirming that the amino terminus of GAD65 does not contribute to the autoepitope and that the IDDM epitope is localised to the middle and carboxyl terminal domains of GAD65. Furthermore, evidence is presented that autoantibodies to GAD65 in IDDM sera react with an epitope formed on a dimeric configuration of the molecule.

L2 ANSWER 8 OF 14 MEDLINE on STN DUPLICATE 5

AB OBJECTIVES: Humoral and cellular immune reactivity are reported for two neuroendocrine autoantigens-glutamic acid decarboxylase (***GAD***) and the protein tyrosine phosphatase IA-2-in a patient with the autoimmune type of stiff-man syndrome and ***insulin*** dependent diabetes (IDDM). METHODS: Antibodies and T cell proliferation against ***GAD*** and IA-2 and cytokine release of antigen stimulated T cells (IFN-gamma) were determined before and several times during immunosuppressive therapy with prednisolone. RESULTS: Raised ***GAD*** antibodies against full length GAD65 or ***chimeric*** constructs were detected before therapy and they remained at a high concentration despite a marked clinical improvement during cortisone treatment. Antibodies to IA-2 were undetectable, but weak T cell responses to both ***GAD*** and IA-2 were seen before therapy and once on reduction of high cortisone dosages when the patient showed signs of clinical deterioration. Cytokine profiles showed increased IFN-gamma production after stimulation with ***GAD*** or IA-2 suggesting increased activation of TH1 cells. CONCLUSION: Immunosuppressive therapy --even with extremely high doses of 500 mg a day--does not lead to the reduction of antibody concentrations in the periphery nor to a switch in epitope recognition of such antibodies despite clinical improvement. The amount of T cell reactivity to various antigens, however, may be a useful marker to monitor the effectiveness of immunotherapy.

L2 ANSWER 9 OF 14 MEDLINE on STN DUPLICATE 6

AB Antibodies to glutamic acid decarboxylase-65 (GAD65) are present in a

number of autoimmune disorders, such as ***insulin*** -dependent (type 1) diabetes mellitus (IDDM), stiff man syndrome, and polyendocrine autoimmune disease. Antibodies to ***GAD*** in IDDM patients usually recognize conformation-dependent regions on GAD65 and rarely bind to the second isoform, glutamic acid decarboxylase-67 (GAD67). In contrast, those present in stiff man syndrome and polyendocrine disease commonly target the second isoform (GAD67) and include antibodies that are less dependent on the conformation of the molecule. By immortalizing peripheral blood B cells with Epstein-Barr virus, we have generated three human IgG autoantibodies, termed b35, b78, and b96, to GAD65 from one patient with multiple autoantibodies to endocrine organs and Graves' disease. All three autoantibodies are of the IgG1 isotype, with islet cell activity, and do not react with GAD67. The regions on GAD65 recognized by the three autoantibodies have been investigated by immunoprecipitation with a series of ***chimeras*** , by binding to denatured and reduced antigens, and using protein footprinting techniques. Using ***chimeric*** ***GAD*** proteins, we have shown that b35 targets the IDDM-E1 region of GAD65 (amino acids 240-435) whereas both b78 and b96 target the IDDM-E2 region of GAD65 (amino acids 451-570). Furthermore, examination of binding to recombinant GAD65 and GAD67 by Western blotting revealed some differences in epitope recognition, where only b78 bound denatured and reduced GAD65. However, b35, b78, and b96 autoantibodies had different footprinting patterns after trypsin treatment of immune complexes with GAD65, again indicating different epitope recognition. Our results indicate that antibodies to GAD65 present in nondiabetic patients with multiple autoantibodies to endocrine organs show similarities to those in IDDM (by targeting IDDM-E1 and IDDM-E2 regions of GAD65) as well as subtle differences in epitope recognition (such as binding to denatured and reduced GAD65 and by protein footprinting). Thus, the GAD65 epitopes recognized by autoantibodies in different autoimmune diseases may overlap and be more heterogeneous than previously recognized.

L2 ANSWER 10 OF 14 MEDLINE on STN DUPLICATE 7

AB The prevalence and titre of epitope-specific autoantibodies to glutamic acid decarboxylase (GAD65) in 155 ***insulin*** -dependent diabetic (IDDM) and 9 GAD65 antibody (Ab)-positive healthy children were determined using four GAD65/67 chimaeric molecules which discriminate among the N-terminal (N), middle (M) and C-terminal (C) epitopes of GAD65. Radioligand binding assays for IgGAb used immunoprecipitation of in vitro translated 35S- ***GAD*** . We found autoantibodies to GAD65 in 116 of 155 (75%), to GAD67 in 19 of 155 (12%) ($p < 0.0001$) and to the GAD65-N-67 chimaera in 25 of 155 (16%) ($p < 0.0001$) IDDM sera. GAD67Ab were found almost exclusively (17 of 19, 89%) in GAD65Ab-positive sera and the levels of GAD67Ab correlated with those of GAD65Ab ($r2 = 0.5913$; $p = 0.009$). GAD65Ab directed to GAD65-M were found in 104 of 155 (67%), to GAD65-C in 104 of 155 (67%) and to GAD65-M + C in 116 of 155 (75%) of IDDM sera, and indicated reactivity to at least two distinct epitopes. Among the nine GAD65Ab-positive healthy children, two (22%) were also positive with GAD67, nine (100%) with GAD65-M + C, seven (78%) with GAD65-M, eight (89%) with GAD65-C and two (22%) with GAD65-N-67. Titres of GAD65Ab ($p = 0.007$), GAD65-C-Ab ($p = 0.002$) and GAD65-C + M-Ab ($p = 0.003$), but not of GAD65-M-Ab ($p = 0.101$) were significantly higher in IDDM than in healthy children. We conclude that GAD65Ab in IDDM and healthy children are directed to middle and C-terminal epitopes, and propose that levels of antibodies specifically directed to the carboxy-terminal end of GAD65 may distinguish IDDM from healthy children.

L2 ANSWER 11 OF 14 MEDLINE on STN DUPLICATE 8

AB Glutamic acid decarboxylase (***GAD***) is an autoantigen in two autoimmune diseases, ***insulin*** -dependent diabetes mellitus (IDDM) and stiff-man syndrome (SMS). However, most individuals with one of these diseases do not have the other disease. Prior studies have suggested that the natures of the ***GAD*** Abs associated with each of these diseases are different, which may have implications for the autoimmune pathogenesis. We have compared the ***GAD*** autoantibody profile and have mapped ***GAD*** protein epitope regions in the two diseases using an immunoprecipitation assay with recombinant ***GAD*** 65 and ***GAD*** 67 proteins, ***GAD*** protein fragments, and synthetic ***GAD*** peptides, as well as ***chimeric*** ***GAD*** proteins. Our results indicate that individuals with SMS have ***GAD*** Abs in 100- to 500-fold higher titer than individuals with IDDM. The population of ***GAD*** Abs in SMS sera is quite complex and includes those that recognize at least three ***GAD*** 65 epitope regions located between amino acids 1-16, 188-442, and 442-563. These types of ***GAD*** Abs are not found in IDDM sera. All SMS sera also had Ab specificity that binds ***GAD*** 67 in a region highly homologous to amino acids 188-442 of ***GAD*** 65. In contrast to prior studies that used immunoblotting to measure ***GAD*** Abs, we find ***GAD*** Abs in SMS sera also target two conformation-dependent regions of ***GAD*** 65, one located in the middle and one near the C-terminus of the protein. These two regions of the ***GAD*** 65 protein are similar to regions targeted by ***GAD*** 65-specific Abs found in individuals with IDDM. These results indicate that although disease-specific epitopes may exist, there is also overlap in the humoral response between the two diseases.

L2 ANSWER 12 OF 14 MEDLINE on STN DUPLICATE 9

AB To study the immune response to glutamic acid decarboxylase (***GAD***

) in ***insulin*** -dependent diabetes mellitus, monoclonal ***GAD*** antibodies after fusion of splenocytes from a nondiabetes-susceptible BALB/c mouse immunized with human recombinant GAD65 were generated. Of the 44 monoclonals, 35 are specific for the GAD65 isoform, whereas 9 also react with GAD67. Some 37 monoclonals, including all GAD65/67 reactive antibodies, react with ***GAD*** by Western blot analysis. The remaining 7 GAD65 monoclonals bind ***GAD*** only in an immunoprecipitation assay, which implies that they target epitopes dependent on the conformation of the ***GAD*** molecule. The 125I-***GAD*** binding of the GAD65 monoclonals reactive on Western blotting was significantly diminished by all 3 sera from Stiff-man syndrome patients but only by 3/30 (10%) sera from type 1 diabetic patients. In contrast, the 7 monoclonal antibodies reactive with a conformation-dependent ***GAD*** epitope were competitive with 83% of ***GAD*** -autoantibody-positive sera from these diabetic patients. Using ***chimeric*** GAD65/67 proteins, the epitope region targeted by these monoclonals was mapped to the middle of GAD65 (amino acids 221-442). This central conformation-dependent ***GAD*** region was also targeted by sera from patients with type 1 diabetes. In conclusion, our data show that even after common immunization of a nondiabetes-susceptible mouse strain, monoclonal were obtained which preferentially react with the GAD65 linear amino-terminus (amino acids 4-17) and a conformation-dependent region located in the middle of ***GAD*** targeted by autoantibodies, indicating that this ***GAD*** region is not restricted to the autoimmune response associated with the Stiff-man syndrome and the beta-cell destruction in type 1 diabetes mellitus.

L2 ANSWER 13 OF 14 MEDLINE on STN DUPLICATE 10
AB Although most individuals with ***insulin*** -dependent diabetes mellitus (IDDM) have autoantibodies to glutamic acid decarboxylase (***GAD***), antibodies to ***GAD*** are also present in some individuals with a low risk of developing diabetes. The ***GAD*** autoantibodies of IDDM are specific for the GAD65 isoform, do not bind denatured ***GAD*** protein, and target epitope(s) dependent on conformation of the protein. However, the IDDM epitopes have been difficult to further define because the antibodies do not bind ***GAD*** protein fragments or synthetic peptides. Since the GAD67 isoform is highly homologous to GAD65 but is usually not a target of the ***GAD*** autoantibodies in IDDM sera, we created six GAD65/GAD67 ***chimeric*** proteins to maintain the overall ***GAD*** protein conformation and used these ***chimeric*** proteins to map conformation-dependent epitopes of GAD65 targeted by IDDM sera. We find that the ***GAD*** binding present in most IDDM sera (n = 11 of 12) is composed of two distinct ***GAD*** antibody specificities that target different conformation-dependent regions of the GAD65 protein, one that is located between amino acids 240 and 435 (termed IDDM-E1) and one that is located between amino acids 451 and 570 (termed IDDM-E2). One IDDM serum (n = 1 of 12) bound only the IDDM-E1 region. Identification of epitopes targeted by IDDM sera may allow one to distinguish between ***GAD*** antibody-positive individuals at high and low risk of developing IDDM and to determine if differences in the autoimmune repertoire directed at ***GAD*** are present. The ***chimeric*** GAD65/GAD67 proteins may also be useful in designing ***GAD*** assays specific for IDDM.

=> d his

(FILE 'HOME' ENTERED AT 13:33:38 ON 22 SEP 2003)

FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 13:33:49 ON 22 SEP 2003
L1 33 S GAD AND INSULIN AND CHIMER?
L2 14 DUPLICATE REMOVE L1 (19 DUPLICATES REMOVED)

=> s gad and b-chain
L3 27 GAD AND B-CHAIN

=> duplicate remove l3
DUPLICATE PREFERENCE IS 'MEDLINE, EMBASE, BIOSIS'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n

PROCESSING COMPLETED FOR L3
L4 12 DUPLICATE REMOVE L3 (15 DUPLICATES REMOVED)

=> d 1-12

L4 ANSWER 1 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:220694 BIOSIS
DN PREV200100220694
TI DNA immunization to treat autoimmune diabetes.
AU von Herrath, Matthias G. (1); Bot, Adrian (1); Whitton, J. Lindsay (1); Coon, Bryan (1)
CS (1) Depts. of Neuropharmacology and Immunology, The Scripps Research Institute, La Jolla, CA, 92037 USA
SO Diabetes-Metabolism Research and Reviews, (January February, 2001) Vol. 17, No. Suppl. 1, pp. S35. print.
Meeting Info.: 5th International Congress of the Immunology of Diabetes Society Madras, Chennai, India February 13-16, 2001
ISSN: 1520-7552.
DT Conference

LA English
SL English

L4 ANSWER 2 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
AN 2000284939 EMBASE
TI 1.alpha.,25-dihydroxyvitamin D3 induces an autoantigen-specific T-helper 1/T-helper 2 immune shift in NOD mice immunized with GAD65 (p524-543).
AU Overbergh L.; Decallonne B.; Waer M.; Rutgeerts O.; Valckx D.; Casteels K.M.; Laureys J.; Bouillon R.; Mathieu C.
CS Dr. C. Mathieu, LEGENDO, Universitair Ziekenhuis, Gasthuisberg, Onderwijs en Navorsing, Herestraat 49, B-3000 Leuven, Belgium. chantal.mathieu@med.kuleuven.ac.be
SO Diabetes, (2000) 49/8 (1301-1307).
Refs: 45
ISSN: 0012-1797 CODEN: DIAEAZ
CY United States
DT Journal; Article
FS 003 Endocrinology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English

L4 ANSWER 3 OF 12 MEDLINE on STN DUPLICATE 1
AN 1999265474 MEDLINE
DN 99265474 PubMed ID: 10334305
TI Cellular immune responses against proinsulin: no evidence for enhanced reactivity in individuals with IDDM.
AU Ellis T; Jodoin E; Ottendorfer E; Salisbury P; She J X; Schatz D; Atkinson M A
CS Department of Pathology, University of Florida College of Medicine, Gainesville 32610, USA.
NC AI-42288 (NIAID)
AI/DK-39250 (NIAID)
DK-45342 (NIDDK)
SO DIABETES, (1999 Feb) 48 (2) 299-303.
Journal code: 0372763. ISSN: 0012-1797.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199906
ED Entered STN: 19990618
Last Updated on STN: 19990618
Entered Medline: 19990610

L4 ANSWER 4 OF 12 MEDLINE on STN DUPLICATE 2
AN 1999264266 MEDLINE
DN 99264266 PubMed ID: 10330296
TI Regulatory Th2-type T cell lines against insulin and ***GAD*** peptides derived from orally- and nasally-treated NOD mice suppress diabetes.
AU Maron R; Melican N S; Weiner H L
CS Brigham and Women's Hospital and Harvard Medical School, Center for Neurologic Diseases, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.
SO JOURNAL OF AUTOIMMUNITY, (1999 Jun) 12 (4) 251-8.
Journal code: 8812164. ISSN: 0896-8411.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS
EM 199906
ED Entered STN: 19990714
Last Updated on STN: 19990714
Entered Medline: 19990629

L4 ANSWER 5 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
AN 2001183732 EMBASE
TI ***GAD*** (65) and insulin ***B*** ***chain*** peptide (9-23) are not primary autoantigens in the type 1 diabetes syndrome of the BB rat.
AU Bieg S.; Hanlon C.; Hampe C.S.; Benjamin D.; Mahoney C.P.
CS C.P. Mahoney, Dept. of Pediatric Endocrinology, Children's Hosp./Regional Med. Ctr., 4800 Sand Point Way NE, Seattle, WA 98105, United States
SO Autoimmunity, (1999) 31/1 (15-24).
Refs: 36
ISSN: 0891-6934 CODEN: AUIMEI
CY United Kingdom
DT Journal; Article
FS 003 Endocrinology
005 General Pathology and Pathological Anatomy
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LA English
SL English

L4 ANSWER 6 OF 12 MEDLINE on STN DUPLICATE 3
AN 1998313782 MEDLINE
DN 98313782 PubMed ID: 9650096
TI Cloned T cells from a recent onset IDDM patient reactive with insulin ***B*** - ***chain*** .
AU Schloot N C; Willemen S; Duinkerken G; de Vries R R; Roep B O
CS Department of Immunohematology, University Hospital Leiden, The Netherlands.
SO JOURNAL OF AUTOIMMUNITY, (1998 Apr) 11 (2) 169-75.
Journal code: 8812164. ISSN: 0896-8411.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS
EM 199810
ED Entered STN: 19981029
Last Updated on STN: 19981029
Entered Medline: 19981022

L4 ANSWER 7 OF 12 MEDLINE on STN DUPLICATE 4
AN 1998313777 MEDLINE
DN 98313777 PubMed ID: 9650091
TI Protection from insulin dependent diabetes mellitus afforded by insulin antigens in incomplete Freund's adjuvant depends on route of administration.
AU Hutchings P; Cooke A
CS Department of Pathology, University of Cambridge, UK.
SO JOURNAL OF AUTOIMMUNITY, (1998 Apr) 11 (2) 127-30.
Journal code: 8812164. ISSN: 0896-8411.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199810
ED Entered STN: 19981029
Last Updated on STN: 19981029
Entered Medline: 19981022

L4 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:367073 BIOSIS
DN PREV199799659006
TI Immunization therapies in the prevention of diabetes.
AU Ramiya, Vijayakumar K.; Lan, Michael S.; Wasserfall, Clive H.; Notkins, Abner L.; Maclaren, Noel K. (1)
CS (1) Dep. Pathol. Lab. Med., PO Box 100275, Univ. Fla., Gainesville, FL 32610-0275 USA
SO Journal of Autoimmunity, (1997) Vol. 10, No. 3, pp. 287-292.
ISSN: 0896-8411.
DT Article
LA English

L4 ANSWER 9 OF 12 MEDLINE on STN DUPLICATE 5
AN 96413835 MEDLINE
DN 96413835 PubMed ID: 8816970
TI Antigen based therapies to prevent diabetes in NOD mice.
AU Ramiya V K; Shang X Z; Pharis P G; Wasserfall C H; Stabler T V; Muir A B; Schatz D A; Maclaren N K
CS Department of Pathology and Laboratory Medicine, University of Florida, Gainesville 32610-0275, USA.
NC R0-1 HD 19469-06 (NICHD)
SO JOURNAL OF AUTOIMMUNITY, (1996 Jun) 9 (3) 349-56.
Journal code: 8812164. ISSN: 0896-8411.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS
EM 199610
ED Entered STN: 19961106
Last Updated on STN: 19961106
Entered Medline: 19961023

L4 ANSWER 10 OF 12 MEDLINE on STN DUPLICATE 6
AN 95407684 MEDLINE
DN 95407684 PubMed ID: 7545875
TI Experimental autoimmune insulitis. Induction by T lymphocytes specific for a peptide of proinsulin.
AU Griffin A C; Zhao W; Wegmann K W; Hickley W F
CS Department of Pathology, Dartmouth Medical School, Lebanon, New Hampshire 03756, USA.
NC NS 27321 (NINDS)
T32 AI 07363 (NIAID)
SO AMERICAN JOURNAL OF PATHOLOGY, (1995 Sep) 147 (3) 845-57.
Journal code: 0370502. ISSN: 0002-9440.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199510

ED Entered STN: 19951026
Last Updated on STN: 19960129
Entered Medline: 19951017

L4 ANSWER 11 OF 12 MEDLINE on STN DUPLICATE 7
AN 93314885 MEDLINE
DN 93314885 PubMed ID: 8100786
TI The 12th International Immunology and Diabetes Workshop. Orlando, Florida.
AU Maclaren N; Lafferty K
CS Department of Pathology and Laboratory Medicine, University of Florida College of Medicine, Gainesville.
SO DIABETES, (1993 Aug) 42 (8) 1099-104.
Journal code: 0372763. ISSN: 0012-1797.
CY United States
DT Conference; Conference Article; (CONGRESSES)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199308
ED Entered STN: 19930820
Last Updated on STN: 19990129
Entered Medline: 19930812

L4 ANSWER 12 OF 12 MEDLINE on STN DUPLICATE 8
AN 94108646 MEDLINE
DN 94108646 PubMed ID: 8281315
TI PDGF-BB exerts trophic activity on cultured GABA interneurons from the newborn rat cerebellum.
AU Smits A; Ballagi A E; Funa K
CS Ludwig Institute for Cancer Research, Biomedical Centre, Uppsala, Sweden.
SO EUROPEAN JOURNAL OF NEUROSCIENCE, (1993 Aug 1) 5 (8) 986-94.
Journal code: 8918110. ISSN: 0953-816X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199402
ED Entered STN: 19940228
Last Updated on STN: 20000303
Entered Medline: 19940215

=> d 1-12 abs

L4 ANSWER 1 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

L4 ANSWER 2 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
AB Prevention of type 1 diabetes in NOD mice by 1,25-dihydroxyvitamin D3 [1.alpha.,25(OH)2D3] is accompanied by a T-helper (Th) 1/Th2 cytokine shift in the pancreas. The aim of this study was to investigate whether this immune shift also occurs outside of the pancreas and whether it is limited to autoantigen-specific immune responses. NOD mice treated with 1.alpha.,25(OH)2D3 (5 .mu.g/kg every 2 days) or control vehicle were immunized with GAD65 (p524-543) or ovalbumin (OVA) in the rear footpads. First, we examined T-cell proliferation and cytokine production (via enzyme-linked immunosorbent assay) of draining lymph node cells in vitro with or without peptide rechallenge. Although no differences in proliferation were measured between control and 1.alpha.,25(OH)2D3-treated mice after in vitro GAD65 rechallenge, a marked shift in cytokine secretion profile was seen in 1.alpha.,25(OH)2D3-treated mice: interleukin-4 was increased (37 .+-. 5 vs. 21 .+-. 12 pg/ml in controls, P < 0.005), whereas .gamma.-interferon levels were decreased (6 .+-. 3 vs. 9 .+-. 3 ng/ml in controls, P < 0.05). This shift was absent in OVA-primed mice. Second, we measured cytokine profiles by reverse transcriptase-polymerase chain reaction in popliteal lymph nodes at different time points after priming with GAD65 or OVA in vivo. A marked Th1/Th2 shift occurred in 1.alpha.,25(OH)2D3-treated mice after in vivo priming with GAD65. Again, this shift was absent after OVA immunization. Finally, we measured cytokine profiles after rechallenge with a panel of autoantigens (GAD65, heat shock protein 65, insulin ***B*** - ***chain***) and control antigens (OVA, keyhole limpet hemocyanine, myelin proteolipid protein, tetanus toxin) and confirmed the Th1/Th2 shift in autoantigen-injected mice but not in control antigen-injected mice. In conclusion, the immune deviation induced by 1.alpha.,25(OH)2D3 in NOD mice can also be induced in the peripheral immune system but is limited to pancreatic autoantigens.

L4 ANSWER 3 OF 12 MEDLINE on STN DUPLICATE 1
AB Investigations of humans and nonobese diabetic mice suggest that proinsulin and/or a fragment of the region spanning C-peptide and the ***B*** - ***chain*** of insulin (i.e., proinsulin peptide) may serve as key autoantigens in IDDM. Therefore, we analyzed cellular immune reactivities against these molecules in people with or at varying risks for the disease to clarify their role in the pathogenesis of IDDM. In vitro peripheral blood mononuclear cell (PBMC) responses against these antigens, a control antigen (tetanus toxoid), and phytohemaglutinin were determined in 60 individuals with newly diagnosed IDDM (< or = 1 day from diagnosis) in 34 islet cell cytoplasmic autoantibody- and/or insulin

autoantibody-negative first-degree relatives of the IDDM subjects, and in 28 autoantibody-negative control subjects. Unlike previous reports suggesting diabetes-associated elevations in cellular immunity to other beta-cell antigens (e.g., ***GAD*** , IA-2, etc.), we observed equivalent levels of phytohemaglutinin stimulation and cellular proliferation in all groups against these antigens (all P values were not significant). The mean stimulation index +/- SD and frequency of reactivity to proinsulin for healthy control subjects and IDDM patients, respectively, were as follows: 1 microg/ml (1.5 +/- 1.0, 1 out of 17 [6%]; 1.9 +/- 1.4, 4 out of 33 [12%]); 10 microg/ml (1.7 +/- 1.3, 1 out of 17 [6%]; 1.2 +/- 0.6, 0 out of 28 [0%]); and 50 microg/ml (1.2 +/- 0.6, 1 out of 16 [6%]; 1.1 +/- 0.6, 1 out of 27 [4%]). The response in healthy control subjects, autoantibody-negative relatives, and IDDM patients, respectively, against the proinsulin peptide fragment were as follows: 1 microg/ml (0.9 +/- 0.4, 1 out of 12 [8%]; 1.3 +/- 1.1, 4 out of 34 [11%]; 1.1 +/- 0.3, 2 out of 28 [7%]); 10 microg/ml (0.9 +/- 0.6, 1 out of 12 [8%]; 1.2 +/- 0.6, 3 out of 34 [9%] 1.4 +/- 1.7, 2 out of 28 [7%]); and 50 microg/ml (1.0 +/- 0.7, 1 out of 12 [8%]; 1.2 +/- 0.5, 2 out of 34 [6%]; 1.3 +/- 0.5, 2 out of 28 [7%]). Taken together with previous studies reporting relatively infrequent occurrences of autoantibodies to proinsulin, the role of immunity to this molecule in the pathogenesis of IDDM in humans remains unclear.

L4 ANSWER 4 OF 12 MEDLINE on STN DUPLICATE 2

AB Non-obese diabetic (NOD) mice spontaneously develop diabetes. Ourselves and others have previously shown that oral and nasal administration of insulin or glutamic acid decarboxylase (***GAD***) suppresses development of diabetes in the NOD mouse and that this suppression appears secondary to the generation of regulatory T cells that act by secreting anti-inflammatory cytokines such as IL-4 and TGF-beta. In the present study, we analysed cytokine patterns associated with mucosal administration of insulin ***B*** - ***chain*** , ***B*** - ***chain*** peptide 10-24 and ***GAD*** peptide 524-543 and derived lines and clones from mucosally-treated animals. Mice were fed five times (400-600 microg/feed) or nasally-treated three times (60 microg/application), and 2 days after the last treatment were immunized in the footpad with the mucosally administered antigen in CFA. Primary immune responses in the popliteal lymph node were measured 10 days after immunization and lines and clones were then established from the primary cultures. There was significantly less IFN-gamma production in mucosally-treated mice associated with increased production of IL-10 and TGF-beta. The nature of the antigen appeared to determine cytokine production as the ***B*** - ***chain*** given either orally or nasally primed for TGF-beta responses, whereas mucosally administered ***B*** - ***chain*** peptide 10-24 primed for IL-10. T cell clones, established from draining lymph nodes of fed or nasally-treated animals, secreted IL-4, IL-10 and TGF-beta whereas those from non-fed mice secreted IL-2 and IFN-gamma. Transfer of Th1 lines with splenocytes from diabetic NOD mice into NOD or NOD/SCID animals accelerated diabetes, whereas transfer of Th2 lines suppressed the development of diabetes. Our results further support a role for Th2-type cells in the regulation of diabetes in NOD mice.

L4 ANSWER 5 OF 12 EMBASE

on STN

AB To investigate whether ***GAD*** (65) whole molecule, ***GAD*** (65) p(35) or insulin ***B*** ***chain*** peptide (amino acids 9-23) play an essential role in the pathogenesis of type 1 diabetes in the BioBreeding (BB) rat, we gave serial injections of ***GAD*** (65), p(35) or insulin ***B*** ***chain*** (9-23) to six groups of BB/Worcester rats. The individual antigens were administered either intrathymically on day 2 and intraperitoneally in MF 59-0 adjuvant 5 times during the first 5 weeks, or by intranasal instillation once neonatally and 5 days/week for the following 6 weeks. Control groups were injected with vehicle only. Age of onset of diabetes and degree of insulitis were not different between controls and antigen-treated rats. Rats that received ***GAD*** (65) intrathymically and intraperitoneally developed high ***GAD*** (65)-antibody titers without altering diabetes development. In ***GAD*** (65)-treated animals, serum antibodies recognized epitopes at 3 sites on ***GAD*** (65) in diabetic animals but only at 1 site in non-diabetic animals. ***GAD*** (65)-injected animals also showed a significant reduction of IFN-.gamma. mRNA expression in the thymus. This study provides evidence against the hypothesis that ***GAD*** (65) and insulin ***B*** ***chain*** peptide (9-23) are primary diabetogenic autoantigens in BB rats because immunizations with these antigens and ***GAD*** (65)-induced immune deviation did not alter the development of diabetes.

L4 ANSWER 6 OF 12 MEDLINE on STN DUPLICATE 3

AB Insulin-dependent diabetes mellitus (IDDM) results from selective autoimmune destruction of insulin producing beta-cells. T-cell reactivity and autoantibodies to several islet proteins such as insulin, ***GAD*** and IA-2 are associated with IDDM in mice and men. In NOD mice, the majority of T cells from insulitis specifically recognize the insulin ***B*** - ***chain*** peptide amino acid 9-22, in contrast to the periphery where the precursor frequency is much lower. It is important to note that these cells are diabetogenic. Surprisingly, the same insulin ***B*** - ***chain*** region contains epitopes recognized by

protective T cells. In fact, autoimmune diabetes in NOD mice could be prevented by prophylactic treatment with this immunodominant T-cell epitope. In humans, however, no immunodominant regions of insulin have yet been defined. We have isolated and characterized a human insulin-specific T-cell clone that was derived from peripheral blood of a newly diagnosed IDDM patient. This patient displayed weakly positive primary T-cell responses to insulin. The peptide recognized by the clone was mapped to the insulin ***B*** ***chain*** (B:11-27). Functionally, the human insulin-specific CD4+ T cells displayed a Th1/0 like cytokine profile and were restricted by HLA-DR. The previously proposed alternative superantigen-like binding of insulin- ***B*** ***chain*** peptide outside of the peptide binding groove of HLA-DR could not be confirmed, since T-cell recognition was inhibited in competition experiments of insulin- ***B*** ***chain*** peptide with HLA-DR16 binding influenza peptide HA307-319. Our results indicate that human clonal T cells isolated from a recent onset IDDM patient recognize an epitope overlapping with the insulin ***B*** - ***chain*** region that is immunodominant and potentially therapeutic in NOD mice. This observation may be useful in studying the role of insulin-specific T cells in IDDM, and may eventually help to establish peptide-based immunotherapies in IDDM.

L4 ANSWER 7 OF 12 MEDLINE on STN DUPLICATE 4

AB Several islet antigens have been shown to modify the time of onset and severity of spontaneous insulin dependent diabetes mellitus (IDDM) in NOD (non-obese diabetic) mice. Oral, intravenous and intra-nasal administration of insulin and glutamic acid decarboxylase (***GAD***) or their derived peptides have all been shown to be effective to differing degrees in reducing the incidence and delaying the onset of diabetes in this mouse model of the disease. Incomplete Freund's Adjuvant (IFA) has also played a key role in tolerance when co-administered with insulin peptides subcutaneously. We show that route of administration may be of crucial importance, since although insulin ***B*** ***chain*** and the B9-23 peptide given in IFA subcutaneously protected (either partially or completely) from IDDM, when given intraperitoneally they completely failed to modify the disease.

L4 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AB Insulin-dependent diabetes (IDD), being an autoimmune disease, offers several opportunities for immunological interventions that may result either in the reduction of disease severity or in delaying diabetes onset. Among the various experimental preventative approaches, parenteral immunization with islet-specific autoantigens appears to be practically simpler and promising. We have previously shown that immunization with insulin, insulin ***B*** ***chain*** and ***B*** ***chain*** epitope (p9-23), but not insulin A chain, in incomplete Freund's adjuvant (IFA) and in alum (with ***B*** ***chain***) delayed/prevented diabetes onset in NOD mice. Here we demonstrate the protective efficacy of affinity purified ***GAD*** -65 in IFA. While both insulin ***B*** ***chain*** and ***GAD*** -65 significantly delayed the onset of diabetes (P=0.001), a recently described tyrosine phosphatase (IA-2) antigen did not (P=0.38). Interestingly, ***B*** ***chain*** immunization reduced the incidence of cyclophosphamide (CY)-accelerated diabetes by about 50-55%. We also provide further evidence that ***B*** ***chain*** , upon increased adsorption to alum, could improve on its protective capacity in NOD mice.

L4 ANSWER 9 OF 12 MEDLINE on STN DUPLICATE 5

AB Interventional approaches that have been successful in delaying insulin-dependent diabetes mellitus (IDDM) using antigen-based immunotherapies include parenteral immunization. It has potential for clinical application provided that effective adjuvants suitable for human use can be found. We have previously shown that immunization with insulin and insulin ***B*** ***chain*** but not A chain in incomplete Freund's adjuvant (IFA) prevented diabetes by reducing IFN-gamma mRNA in the insulitis lesions. In this paper we show that the insulin ***B*** ***chain*** peptide (p9-23) contain the most protective epitope. Immunization with selected ***GAD*** peptides was ineffective. Immunization with ***B*** ***chain*** but not A chain using alum as adjuvant delayed diabetes onset (P = 0.012), whereas administration of alum alone was not protective. When Diphtheria-Tetanus toxoid-Acellular Pertussis (DTP) vaccine was used as the adjuvant vehicle, DTP itself induced significant protection (P < 0.003) which was associated with a Th2-like cytokine producing insulitis profile, IL-4 driven IgG1 antibody responses to insulin, ***GAD*** in the periphery and an augmentation of the autoimmune response to ***GAD*** . The anti-diabetic effect of DTP was enhanced when given with insulin ***B*** ***chain*** . These results encourage consideration of an approach using alum/DTP and insulin ***B*** ***chain*** immunization in clinical trials.

L4 ANSWER 10 OF 12 MEDLINE on STN DUPLICATE 6

AB Type I diabetes, an autoimmune disease that occurs in humans and animals, is characterized by the destruction of insulin-secreting islet beta-cells of the pancreas. Antibodies directed toward multiple islet protein can be detected before diagnosis of type I diabetes; however, the identity of the inciting autoantigen(s) that targets beta-cells for destruction has not been defined. Autorecognition of many self-proteins by CD4+ T lymphocytes is restricted by the products of class II immune response genes of the major histocompatibility complex (MHC), and in human type I diabetes such

a MHC association has been described. The present study uses a rat MHC class II (RT1.Bl) peptide binding motif to predict potentially autoreactive CD4+ T cell epitopes in two key islet beta-cell constituents: the enzyme glutamic acid decarboxylase (***GAD***) and the insulin precursor hormone proinsulin (PI). Seventeen-amino-acid-long peptide fragments of ***GAD*** and PI containing the binding motif were synthesized and used to generate peptide-specific, MHC class II-restricted, CD4+ T cell lines. Once established, the T cell lines specific for rat islet ***GAD*** and PI were adoptively transferred to naive, MHC-compatible rats. At 10 days after transfer, insulitis had developed in rats receiving PI-specific T cells, whereas no insulitis was observed in pancreata of rats receiving ***GAD*** -specific T cells. Of particular interest is the finding that the pathogenic T cell epitope identified in PI spans the endogenous cleavage site between the ***B*** - ***chain*** and C-peptide of insulin. Moreover, the PI-specific T cells were able to react specifically with material produced in vitro by a rat insulinoma cell line. These results demonstrate that pathogenic T cell epitopes can be located in portions of molecules that are subsequently degraded during normal enzymatic processing. As PI is found highest concentrations in the beta-cells of pancreatic islets, it is possible that this molecule and not its individual degradation products (ie, insulin and C-peptide) might serve as an autoantigen in the pathogenesis of type I diabetes.

L4 ANSWER 11 OF 12 MEDLINE on STN DUPLICATE 7
AB The 12th International Immunology of Diabetes Workshop was held during April 1993 in Orlando, Florida, to review research progress since the 11th Immunology of Diabetes Workshop meeting in Nagasaki, Japan, one and a half years before. The NOD mouse may have as many as 10 susceptibility genes, including its novel IA major histocompatibility complex antigen and a defective interferon-gamma receptor, whereas human IDDM is so far known to be encoded by cis and trans complementation products of certain DQ genes on chromosome 6q, and a gene in the insulin-like growth factor II region on chromosome 11p. A unique protein regulator of the X box promotor of the highly susceptible DQB1*0302 allele has also been found. Islet cell antibody negative siblings of IDDM patients appear to have lower than expected abilities to secrete insulin in response to intravenous glucose. Sera from patients before and/or after developing IDDM immunoprecipitate two native proteins of 64,000- and 38,000-M(r) glutamic acid decarboxylase (GAD65) reacting to conformational epitopes. However, a multitude of other autoantibodies often reacting to denatured proteins through linear epitopes have also been identified. The first workshop for ***GAD*** antibody assays was successfully completed; however, the 38,000-M(r) antigen has not yet been identified. Other autoantibodies reactive to gangliosides and to sulfatides continue to be reported. Insulitis has come to be recognized as a sometimes protective event. Protective insulitis predominates in older lesions. It can be induced by as disparate means as tuberculin antigen administration, by interleukin-4 treatments, by transfer of T-cell lines generated in autologous mixed lymphocyte responses, and by immunization to insulin ***B*** - ***chain*** , whereas oral islet cell antigens, such as insulin, can delay diabetes onset in the NOD mouse.(ABSTRACT TRUNCATED AT 250 WORDS)

L4 ANSWER 12 OF 12 MEDLINE on STN DUPLICATE 8
AB Platelet-derived growth factor (PDGF) is a well known mitogen for mesenchyme-derived cells and glial cells. Its presence in neuronal cells of the central nervous system has only recently been described. We have shown earlier that neurons of newborn rat brains in culture express PDGF beta-receptors and that PDGF-BB, a homodimer of PDGF ***B*** - ***chain*** , increases survival and promotes neurite outgrowth of newborn cerebellar cells (Smits et al., Proc. Natl Acad. Sci. USA, 88, 8159-8163, 1991). In this study, the effects of PDGF on early postnatal rat cerebellar cells were further explored. By using chemically defined serum-free medium, we have established primary cell cultures of rat cerebella (postnatal day 4-5) containing 70-80% neuronal cells. During the first 10 days in vitro, no difference in total cell number was found between PDGF-BB-treated and untreated cultures. After this time period, however, increased survival of the PDGF-BB-treated cells was found. Within the first 10 days in vitro, the addition of PDGF-BB to the cultures resulted in a relative increase in survival of interneurons expressing glutamic acid decarboxylase (***GAD***), the GABA biosynthetic enzyme. Moreover, addition of PDGF-BB in the untreated cell culture resulted in a rapid increase of ***GAD*** mRNA. These results show that PDGF-BB acts as a trophic factor on GABAergic interneurons of the cerebellum by up-regulating ***GAD*** synthesis and prolonging the survival of these cells. Furthermore, in situ hybridization revealed that there are scattered cells present in the early postnatal cerebellum that express PDGF beta-receptor mRNA.(ABSTRACT TRUNCATED AT 250 WORDS)

=> d 9-10

L4 ANSWER 9 OF 12 MEDLINE on STN DUPLICATE 5
AN 96413835 MEDLINE
DN 96413835 PubMed ID: 8816970
TI Antigen based therapies to prevent diabetes in NOD mice.
AU Ramiya V K; Shang X Z; Pharis P G; Wasserfall C H; Stabler T V; Muir A B; Schatz D A; Maclaren N K
CS Department of Pathology and Laboratory Medicine, University of Florida,

Gainesville 32610-0275, USA.
NC R0-1 HD 19469-06 (NICHD)
SO JOURNAL OF AUTOIMMUNITY, (1996 Jun) 9 (3) 349-56.
Journal code: 8812164. ISSN: 0896-8411.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS
EM 199610
ED Entered STN: 19961106
Last Updated on STN: 19961106
Entered Medline: 19961023

L4 ANSWER 10 OF 12 MEDLINE on STN DUPLICATE 6
AN 95407684 MEDLINE
DN 95407684 PubMed ID: 7545875
TI Experimental autoimmune insulitis. Induction by T lymphocytes specific for a peptide of proinsulin.
AU Griffin A C; Zhao W; Wegmann K W; Hickley W F
CS Department of Pathology, Dartmouth Medical School, Lebanon, New Hampshire 03756, USA.
NC NS 27321 (NINDS)
T32 AI 07363 (NIAID)
SO AMERICAN JOURNAL OF PATHOLOGY, (1995 Sep) 147 (3) 845-57.
Journal code: 0370502. ISSN: 0002-9440.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199510
ED Entered STN: 19951026
Last Updated on STN: 19960129
Entered Medline: 19951017

=> d 8

L4 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1997:367073 BIOSIS
DN PREV199799659006
TI Immunization therapies in the prevention of diabetes.
AU Ramiya, Vijayakumar K.; Lan, Michael S.; Wasserfall, Clive H.; Notkins, Abner L.; Maclaren, Noel K. (1)
CS (1) Dep. Pathol. Lab. Med., PO Box 100275, Univ. Fla., Gainesville, FL 32610-0275 USA
SO Journal of Autoimmunity, (1997) Vol. 10, No. 3, pp. 287-292.
ISSN: 0896-8411.
DT Article
LA English

=> d his

(FILE 'HOME' ENTERED AT 13:33:38 ON 22 SEP 2003)

FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 13:33:49 ON 22 SEP 2003
L1 33 S GAD AND INSULIN AND CHIMER?
L2 14 DUPLICATE REMOVE L1 (19 DUPLICATES REMOVED)
L3 27 S GAD AND B-CHAIN
L4 12 DUPLICATE REMOVE L3 (15 DUPLICATES REMOVED)

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COST IN U.S. DOLLARS	ENTRY	SINCE FILE SESSION	TOTAL
FULL ESTIMATED COST		42.31	42.52

STN INTERNATIONAL LOGOFF AT 13:45:35 ON 22 SEP 2003